



TITLE:

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Determination of the Major Antigenic Components of Mite
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Non cross-reactivity between the major antigenic
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SUMMARY

The antigenic components of mite (*Dermatophagoides farinae*, Df) and house dust (HD) allergen were investigated by the Western blotting method. The major antigens were examined with respect to the frequency of binding of the specific IgE antibodies from asthmatic sera. The major antigens of Df had Mr of 16, 24, 28, and 90kD, and the major HD antigen had a Mr of 25kD. Besides having apparently different molecular sizes, these components also had no antigenic cross-reactivity. Therefore, our HD allergen was thought to have the major antigen that has distinct antigenicity from mite allergen. However, we could not clarify the source of our HD major antigen.

INTRODUCTION

Mite allergens, of both *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp) have been considered to be the major antigenic components of house dust (HD) allergens¹⁻⁷. These mite allergens are therefore called "house dust mite" allergens. Previous studies examined the relationship between the two allergens. Measurement of the number of mites contained in house dust¹, skin reactions²⁻⁵, provocation tests², immunodiffusion⁷, gel filtration²⁻⁶, or radio-immunoassays⁶, indicated that mites were indeed the major antigen of HD. These findings, however, are inconclusive in determining the unique antigen of the two allergens, since these studies were designed to determine the antigen common to the two allergens.

Moreover, we often encounter asthmatic patients with high RAST scores against Df and/or Dp allergens, and low or negative RAST scores against the HD allergen. This suggests that mite allergens are not the only major antigenic component of HD allergen.

In the present study, we first examined the major antigens of Df and HD, and second, the relationship between Df and HD allergens.

MATERIALS AND METHODS

Atopic sera and anti-sera

Sera from 26 asthmatic patients, and 22 healthy volunteers were used. All sera from the

asthmatic patients had high RAST scores (more than class 3) against the Df allergen, whereas only 10 had high RAST scores against the HD allergen.

The sera from the healthy volunteers had negative RAST scores (less than class 1) against the Df and HD allergens.

Goat anti-human IgE, peroxidase conjugated swine anti-goat IgG antibodies were obtained from TAGO Inc. (Burlingame, CA. USA). All these anti-sera had previously been purified through affinity chromatography, and the swine anti-goat IgG antibody had additionally been absorbed by human serum. The specificities of the anti-sera were tested before use.

Allergens

The Df allergen extracted from mite bodies cultured without human dander, and the HD allergen were obtained from Torii & Co. Ltd. (Tokyo, Japan). The Dp allergen was kindly provided by Dr. Hosoi⁸ (Department of Pediatrics, Kyoto University). The protein concentrations of these allergens are as follows: for Df, 45 μ gPN/mg lyophilized powder; for HD, 10 μ gPN/mg lyophilized powder; (Dp was not examined). Powdered Df allergen was prepared into a 4 mg/ml solution and HD allergen into an 18 mg/ml solution to adjust the protein concentrations. Powdered Dp allergen was also prepared into a 4 mg/ml solution.

Except in some experiments, each allergen was pretreated with 1% sodium dodecylsulfate (SDS) and 10% 2-mercaptoethanol (2ME) at a temperature of 100°C for 5 minutes before use.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrical transfer of allergens

SDS-PAGE was carried out under the conditions described by Laemmli⁹. In brief, 3.6 μ gPN of Df and HD allergens were placed on a 12.5% slab of SDS-polyacrylamide gel, together with 80 μ g powder/20 μ l solution of Dp allergen. They were then subjected to electrophoresis at 40 mA for 5 hours. A low molecular weight marker (Pharmacia, Uppsala, Sweden) was used to measure the relative molecular weight (M_r) of the fractionated allergens. In some experiments, the slab of gel was directly stained after electrophoresis with 0.5% Coomassie brilliant blue dye to detect the fractionated protein components of these allergens. After electrophoresis, the slab of gel was fixed adjacent to the nitrocellulose membrane (Schleicher & Schüll GmbH, W. Germany), and placed in an electroblotting device (Toyo Filterpaper Inc. Tokyo, Japan). The fractionated allergens were thus electrically transferred from the SDS-polyacrylamide gel onto the nitrocellulose membrane¹⁰. The blotting buffer contained a 25 mM tris base, 192 mM glycine and 20% ethanol (pH 8.3). Electroblotting was carried out at 30 V for 18 hours. The temperature of the blotting buffer was kept at 4°C by a refrigerated circulating bath.

In some experiments, following electroblotting, the gel and nitrocellulose membrane were stained by 0.1% Amide black to ascertain whether the electroblotting had been carried out sufficiently.

Enzyme immunostaining assays

Enzyme immunostaining assay was carried out using a slight modification of the horseradish peroxidase procedure described by Hawks¹¹. In brief, following SDS-PAGE and electroblotting, we blocked the remaining active sites of the nitrocellulose membrane with 3% gelatin in tris buffered saline (TBS; 50 mM tris HCl, 200 mM NaCl, pH 7.5) for 1 hour. Then, the

membrane was cut into strips, incubated with test sera diluted 1 : 5 in 1% gelatin TBS for 3 hours, and shaken gently. To detect any antigenic components that reacted with specific IgE antibodies, the strip was incubated with goat anti-human IgE (1 : 100) shaken gently for 1 hour. This was followed by incubation with peroxidase-conjugated swine anti-goat IgG (1 : 100) for 1 hour.

After these procedures, the strips were incubated with 0.025% solution of 3, 3'-diaminobenzidine containing 0.04% H₂O₂ for 5-10 minutes for color development. These procedures were carried out at room temperature, and each procedure was followed by extensive rinsing of the membrane in 0.05% Tween 20- TBS.

Affinity chromatography

Cyanogen bromide (CNBr) activated Sepharose 4B (Pharmacia, Uppsala, Sweden) was coupled with antigens and used in the same way as described by Tan Wilson¹². In brief, 2 ml of gelled Sepharose 4B was incubated with each allergen at concentrations of 2 mg powdered mites and 3.6 mg powdered HD in a coupling buffer (0.5 M NaCl, 0.1 M NaHCO₃, pH 8.3) at a temperature of 4°C for 72 hours. The remaining active sites of Sepharose 4B were blocked with 1 M monoethanol amine pH 8.0 for 24 hours followed by extensive washing with 0.1 M acetate buffer pH 4.0 and coupling buffer alternatively. This procedure was repeated several times. Then 2 ml of test serum was added to each 2 ml of allergen-coupled Sepharose 4B, incubated at 4°C for 24 hours in a 5 ml silicone test tube, and centrifuged at 1200 rpm for 10 minutes. This procedure was carried out three times and the supernatant was used as absorbed serum. The absorbed serum was tested for enzyme immunostaining assays in the same way as described above.

RESULTS

Fractionation of Df, Dp and HD allergens through SDS-PAGE

As shown in Fig. 1, Df allergen showed visible bands of Mr 13.3, 14, 16, 18, 22, 24, 28, 32, 37, 45, 60, 68, 70, 90 and 100 kilo-daltons (kD). The Dp allergen was found to have fractions similar to those of the Df allergen, but the HD allergen had components of Mr 15, 28, 60 and 70kD. Two-ME treated allergens were composed of almost similar components to 2ME non-treated allergens.

Detection of the major antigenic components of Df allergen

We discovered that Df-specific IgE antibodies bind to the components of Mr 14, 16, 24, 28, 32, 60, 70 and 90kD (Fig. 2).

Fig. 3 shows the frequencies of binding of IgE antibodies against these components. The component with a Mr of 16kD was antigenic in 100% (24/24 cases) of sera with regard to IgE antibodies. The component with a Mr of 24kD bound IgE antibodies in 33% (8/24), that of Mr 28kD bound in 30% (7/24) and that of Mr 90kD in 25% (6/24) of the asthmatic cases. Other components bound IgE antibodies at lower frequencies than these four components. Thus, the component with a Mr of 16kD was considered to be the major antigen of Df.

The sera from healthy volunteers did not bind any component of Df allergen with respect to IgE antibodies.

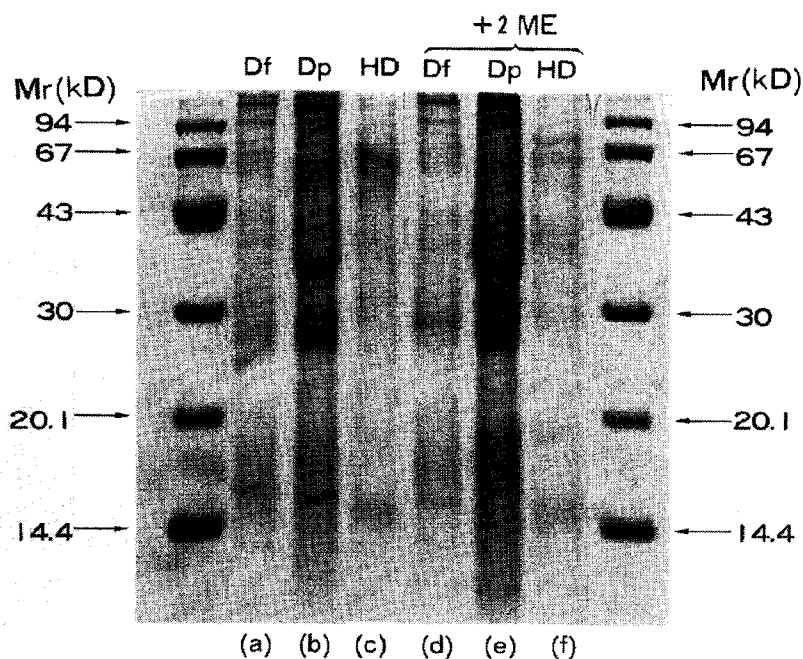


Fig. 1 Fractionated Df (lane a, d), Dp (b, e) and HD (c, f) allergens through 12.5% SDS-PAGE, with and without 2ME pretreatment. Stained with 0.5% Coomassie brilliant blue. Molecular weight marker (both sides), including; phosphorylase B (94kD), bovine serum albumin (67kD), ovalbumin (43kD), carbonic anhydrase (30kD), soybean trypsin inhibitor (21.1kD), and α -lactalbumin (14.4kD) from the top to bottom.

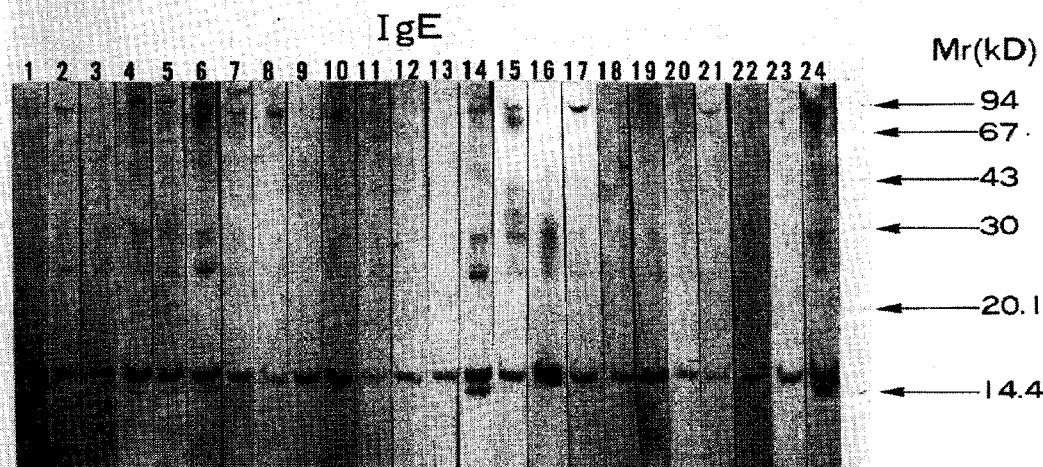


Fig. 2 The enzyme immunostaining of Df specific IgE reactive components of fractionated Df allergen, using 24 atopic sera with positive RAST scores against Df allergen. Molecular weights are shown on the right side.

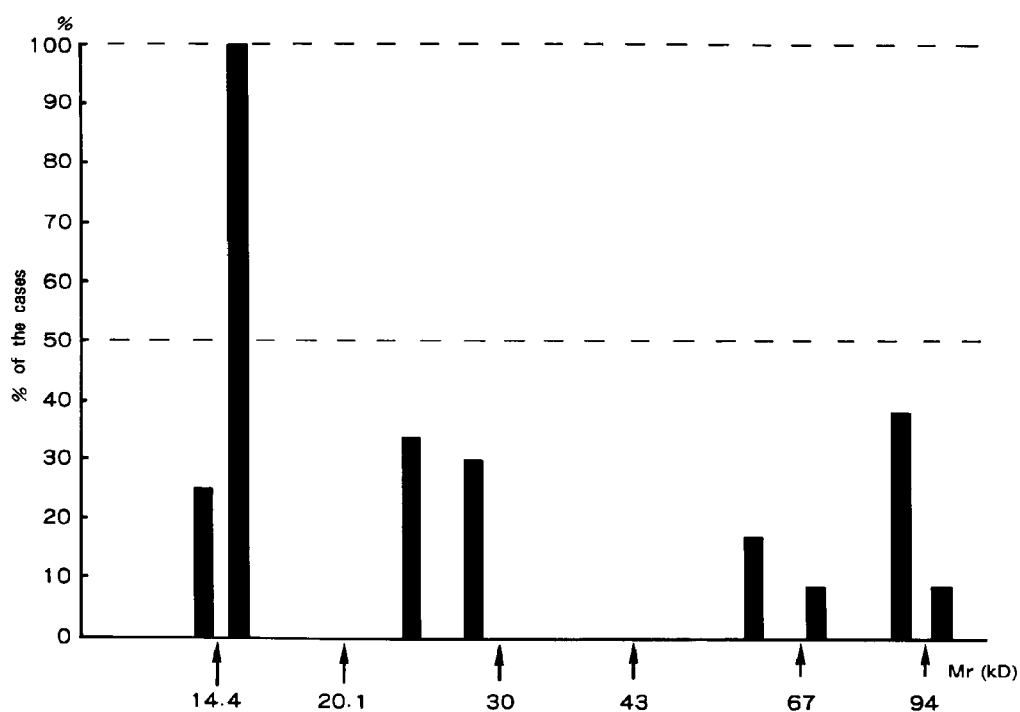


Fig. 3 Frequencies of binding of Df specific IgE antibodies against fractionated Df allergen in 24 atopic sera

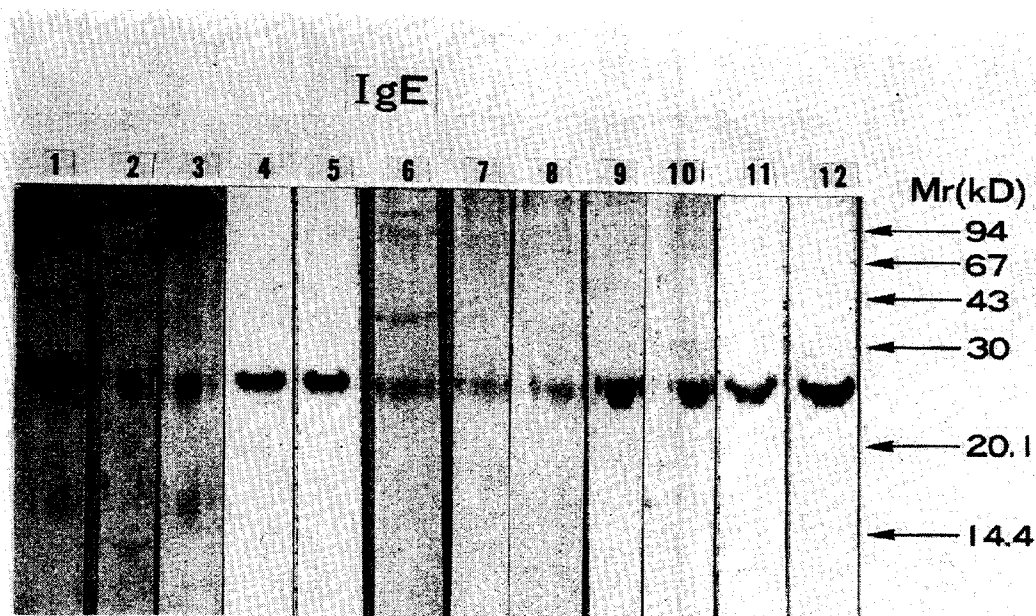


Fig. 4 The enzyme immunostaining of HD specific IgE reactive components of fractionated HD allergen, using 12 atopic sera with positive RAST scores against HD allergen.

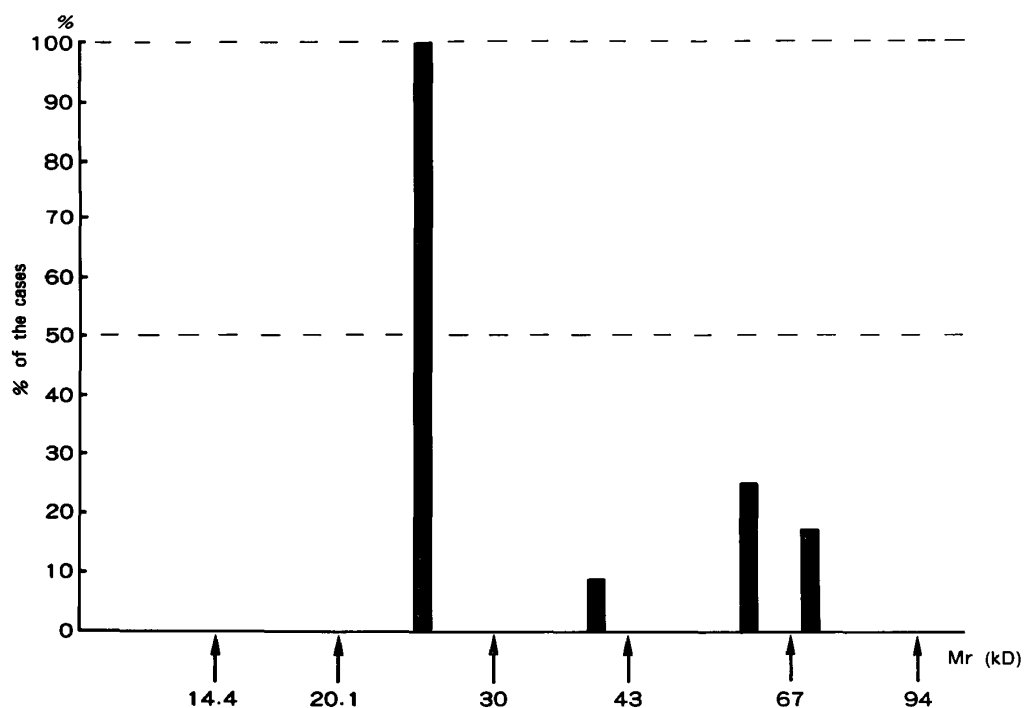


Fig. 5 Frequencies of binding of HD specific IgE antibodies against fractionated HD allergen in 12 atopic sera.

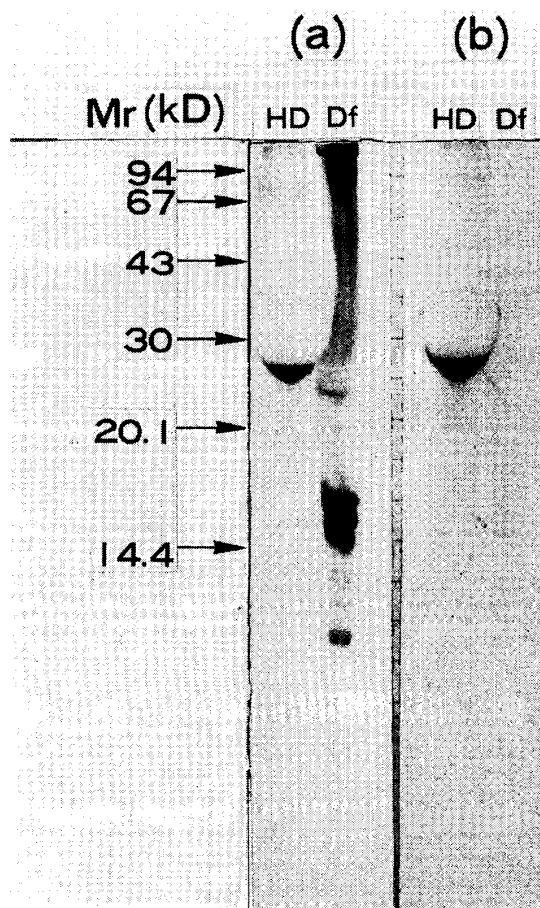


Fig. 6 Alteration of the reactivity of atopic serum (with high reactivities against either Df, and HD allergens) before (a) and after (b) incubation with Sepharose 4B coupled Df allergen. In brief, 2ml of test serum was incubated with 2mg of Df allergen coupled on 2ml gel of Sepharose 4B for 24 hours. This procedure was carried out three times and the supernatant was used as an absorbed serum.

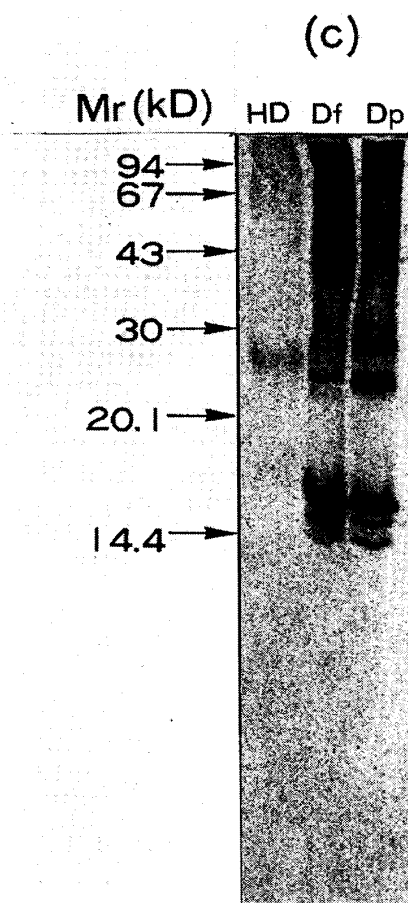


Fig. 7 Enzyme immunostaining of the fractionated Df, Dp and HD allergens. These three allergens were simultaneously electrophoresed through 12.5% SDS-polyacrylamide gel, transferred onto the nitrocellulose membrane.

Detection of the major antigenic components of HD allergen

Although the 2ME treated HD allergen produced only four visible bands when stained with Coomassie brilliant blue dye, the enzyme immunostaining assay revealed that the major antigenic component of HD allergen was of Mr 25kD, which was antigenic in 100% (12/12) of the sera from asthmatic patients (Fig. 4 and 5). This component could not be stained by Coomassie brilliant blue (Fig. 1) or Amide black 10B dye (data not shown), and could be detected by immunostaining assay only when the HD allergen had been pretreated with 10% 2ME (data not shown);

Other components with a Mr of 40kD (8%, 1/12), 60kD (25%, 3/12) and 70kD (17%, 2/12) were also found to bind specific IgE antibodies.

No IgE binding component was found in 5 control sera. Therefore, the component with a Mr of 25kD was thought to be the major antigen of HD.

Non cross-reactive antigenicities between Mite and HD major antigenic components

To determine whether there are cross-reactivities between this Mr 25kD HD component and Mr 16kD Df component, or whether this Mr 25kD HD component is identical to the Mr 24kD or 28kD Df components, absorption studies were performed. The sera used in these experiments had high reactivities against both mite and HD allergens with regard to IgE anti-

bodies. As can be seen in Fig. 6, the component of Df with a Mr of 16kD, and that of HD with a Mr of 25kD had two distinct antigenicities. Also the component of Df with a Mr of 24kD or 28kD, and that of HD with a Mr of 25kD have different antigenicities. Additionally, Fig. 7 shows that there are slight differences in the molecular sizes of these components : ie, Mr 24 or 28kD of Df, and Mr 25kD of HD.

DISCUSSION

We investigated whether there are differences in the antigenicities among the major antigenic components of Df and HD allergens, by using the Western blotting method.

The frequencies of binding of Df-specific IgE antibodies against the fractionated Df allergen were investigated, and four components were found to have relatively high frequencies of binding. These components had a Mr of 16kD (100% of cases), 24kD (33%), 28kD (30%), and 90kD (25%). The molecular weights of these antigenic components suggest that some of them correspond to the previously described standardized mite allergens. Our 16kD Df component may correspond to antigen DpX (16kD)¹³, and the 24kD component may also correspond to the antigen P1¹⁴. However, the component of Df with a Mr 24kD (which has an identical molecular weight to antigen P1) showed a lower frequencies of binding than the component of Df with a Mr 16kD (which also has identical molecular weight to antigen DpX). The source of the antigen P1 is thought to be mite debris (particularly the mite fecal particles), whereas the antigen DpX is thought to be of mite body origin. Since our Df allergen is extracted from cultured mite bodies, it is not surprising that the Df component of Mr 16kD was more frequently antigenic than the Mr 24kD component of Df.

On the other hand, the frequencies of binding of HD-specific IgE antibodies against fractionated HD allergen show that the component with a Mr of 25kD is the major antigen of HD. This component was different from the Mr 16, 24, and 28kD components of Df in molecular size. Absorption studies also revealed that there are no common antigenicities or cross-reactivities among the major antigens of HD and Df. This finding is in conflict with the previous finding, in which the major antigen of HD is of mite origin.

Recently, however, some investigators concluded that the major antigenic components of commercial house dust allergens were of cat or dog origin, and that the contents of mites are relatively low¹⁵⁻¹⁷. Therefore, it is not surprising that our HD allergen contains a major antigenic component that bears different antigenicities from mite allergen.

We did not compare the antigenicity of our HD to the cat or dog allergens. Therefore, whether our HD allergen shares antigenicities with the cat or dog allergens should be further investigated. However, it is clear that our HD allergen, which is most commonly used in Japan for skin-testing and for hyposensitization, contains the major antigen that bears different antigenicity from the mite allergen.

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